

**Standard Operating Procedure
for Nitrate - Nitrite in Lake Water (QuikChemFIA+8000
Method)**

LG203

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Standard Operating Procedure for Nitrate - Nitrite in Lake Water (QuikChemFIA+8000 Method)

1.0 SCOPE AND APPLICATION

- 1.1 This method covers the determination of nitrate and nitrite in lake water.
- 1.2 The approximate working range is 0.03 to 2.00 mg N (as $\text{NO}_3 + \text{NO}_2$)/L. The method detection limit is 0.03 mg N/L.

2.0 SUMMARY

- 2.1 Nitrate is quantitatively reduced to nitrite by passage of the sample through a column containing copper coated cadmium.
- 2.2 The nitrate (reduced nitrate plus original nitrite) is determined by diazotizing with sulfanilamide dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm.

3.0 SAMPLE HANDLING AND PRESERVATION

- 3.1 Samples are collected in clean plastic containers with polypropylene caps.
- 3.2 Samples are preserved by addition of 1 mL of concentrated sulfuric acid per liter of sample.

4.0 INTERFERENCES

- 4.1 Residual chlorine can interfere by oxidizing the cadmium column.
- 4.2 Low results would be obtained for samples that contain high concentrations of iron, copper, or other metals. In this method, EDTA is added to the buffer to reduce this interference.

5.0 APPARATUS

- 5.1 13 x 100 mm test tubes
- 5.2 Lachat QuikChem FIA+8000
 - 5.2.1 XYZ Sampler
 - 5.2.2 Nitrate/Nitrite Manifold (Lachat Method # 10-107-04-1-C)
 - 5.2.3 Cadmium-Copper Reduction Column
 - 5.2.4 Printer

6.0 REAGENTS AND STANDARDS

6.1 All reagents should be stored in the appropriate bottles and labeled with the following information:

Identity: (15 N Sodium hydroxide)
Concentration: (1000 mg N/L)
Date of Preparation: (mm/dd/yy)
Expiration Date: (mm/dd/yy)
Initials of Preparer: (IMF)

6.2 Use reagent water for all solutions.

6.3 **15 N Sodium Hydroxide:** Add 150 g of NaOH very slowly to 250 mL of reagent water.

CAUTION: *The solution will get very hot!* Swirl until dissolved. Cool and store in a plastic bottle.

6.4 **Ammonium Chloride buffer, pH 8.5:** In a 1-L volumetric flask, dissolve 85.0 g ammonium chloride (NH_4Cl) and 1.0 g disodium ethylenediamine tetraacetic acid dihydrate ($\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$) in about 800 mL of reagent water. Adjust pH to 8.5 with 15 N NaOH. Degas with helium.

An alternative recipe for Ammonium Chloride buffer is:

In a hood, to a 1-L volumetric flask, add about 500 mL of reagent water, 105 mL concentrated hydrochloric acid (HCl), 95 mL ammonium hydroxide (NH_4OH) and 1.0 g disodium EDTA ($\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$). Dissolve and dilute to the mark. Invert to mix. Adjust the pH to 8.5 with HCl or 15 N NaOH solution. Degas with helium.

6.5 **Sulfanilamide color reagent:** To a 1-L volumetric flask add about 600 mL of reagent water. Then add 100 mL of 85% phosphoric acid (H_3PO_4), 40.0 g sulfanilamide, and 1.0 g N (1-naphthyl) ethylenediamine dihydrochloride (NED). Shake to wet, and stir to dissolve. Dilute to the mark, and invert to mix. Degas with helium. Store in a dark plastic bottle. This solution is stable for one month.

6.6 Preparation of Standards

6.6.1 **Stock Calibration Standard (1000 mg N/L):** Dissolve 7.218 g of potassium nitrate (KNO_3), dried for 1 hour at 105°C , in 500 mL of reagent water. Add 1 mL of concentrated sulfuric acid and dilute to 1 L.

6.6.2 **Intermediate Calibration Standard (100 mg N/L):** Transfer 100 mL of Stock Calibration Standard (6.6.1) into a 1-L volumetric flask, add 1.0 mL of concentrated sulfuric acid and dilute to 1 L with reagent water.

6.6.3 **Working Standards:** Prepare standards over the range of analysis. For the working range of 0 - 2.00 mg N/L, the following standards may be used:

mL of Intermediate Calibration Standard (6.6.2) diluted to 1 L	Concentration in mg of N/L
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0.0	0.00
0.2	0.02
2.5	0.25
5.0	0.50
7.5	0.75
10.0	1.00
20.0	2.00

NOTE: Use volumetric flasks. Preserve the working standards by addition of 1 mL of concentrated sulfuric acid.

6.6.4 **Stock Control Standard (100 mg N/L):** Any nitrate compound may be used for control standards. Someone other than the analyst should prepare the control standards. Weigh 0.6068 g of sodium nitrate (NaNO_3) (dried at 105°C for one hour). In a 1-L volumetric flask with approximately 500 mL of reagent water dissolve this amount of sodium nitrate, add 1 mL concentrated H_2SO_4 and dilute to mark.

6.6.5 Prepare the control standards using solution (6.6.4).

Standard ID	mL Stock Control Standard (6.6.4) diluted to 1 Liter	Concentration mg-N/L
High Check Standard (CH)	6	0.600
Low Check Standard (CL)	2	0.200

NOTE: Use volumetric flasks. Preserve the control standards by addition of 1 mL of concentrated sulfuric acid.

7.0 PROCEDURE

7.1 Follow the Lachat Procedural SOP (Typical Daily Operation Section).

7.2 Remember to establish reagent flow through entire system before diverting flow through cadmium column.

8.0 CALCULATIONS

8.1 The computer yields results directly in mg N (as $\text{NO}_2 + \text{NO}_3$)/L.

9.0 QUALITY CONTROL

9.1 Refer to the Chapter 2 Introduction for definitions of quality control samples and information regarding quality control procedures, such as QC sample IDs and labeling.

9.2 Instrument Calibration and Frequency

Calibration should be conducted as described in this SOP and in Section 13 of Appendix B of this manual. QC check standards, such as high check and low check standards, must be prepared from a separate source as the control standards.

9.3 Method Detection Limits

Method detection limit (MDL) studies should be performed according to 40 CFR, part 136, Appendix B (if applicable), or by another procedure that is pre-approved by the WQS QM Technical Lead or the GLNPO QM. MDL studies must be conducted once per year and each time a significant change is made to the analytical SOP, including changes in instrumentation.

- 9.4 The following QC samples must be prepared and analyzed at the minimum frequency indicated. External QC samples are collected and prepared by GLNPO during the survey and internal QC samples are prepared by the contractor laboratory.

QC Sample Type		Frequency	Acceptance Criteria
External	Field Reagent Blank (FRB)	One per basin ^a	0.00 ± 0.03 mg/L or less than one-tenth associated field sample concentrations, whichever is greater
	Field Duplicate (FD1)	One per basin ^a	Relative Percent Difference (RPD) ≤ 20%
	Lab Duplicate (LD1)	One per basin ^a	Relative Percent Difference (RPD) ≤ 20%
Internal	Calibration	At the beginning of each batch	r ≤ 0.995
	High Check Standard (CH)	At the beginning & end of each batch or 1 per 40 samples, whichever is more frequent	0.60 ± 0.09 mg/L
	Low Check Standard (CL)	At the beginning & end of each batch or 1 per 40 samples, whichever is more frequent	0.20 ± 0.03 mg/L
	Laboratory Reagent Blank (LRB)	At the beginning & end of each batch or 1 per 40 samples, whichever is more frequent	0.00 ± 0.03 mg/L or less than one-tenth associated field sample concentrations, whichever is greater
	Method Detection Limit (MDL)	Once per year and each time a significant change is made to the SOP	0.03 mg/L

^a A field duplicate, lab duplicate, and field reagent blank are collected with each group of 3, 4, or 5 stations depending on the lake. A Random Number Generator (RNG) is used to determine the stations and depths of these QC samples. Where basins are well defined, at least one of each is collected from each basin.

9.5 Assessment

- 9.5.1 The analyst must compare analytical results to the acceptance criteria listed in Section 9.2 to identify QC failures. If the results are outside the acceptance criteria, the analyst should first review their calculations for errors and if none are identified, they must follow the corrective action procedures listed in Section 9.6.

9.6 Corrective Actions

- 9.6.1 Corrective action procedures will often be handled at the bench level by the analyst, who reviews the preparation or extraction procedure for possible errors, checks the instrument calibration, spike and calibration mixes, instrument sensitivity, and any other potential sources of error. If failure occurs and an error is identified, the analyst should re-run quality control and RFS samples in the entire analytical batch to confirm the results. Because external QC samples are collected and prepared during the survey and provided to the contractor or grantee laboratory, a single rerun to confirm results is sufficient when all other QC samples are within acceptance criteria. For analyses conducted onboard, if the problem persists or cannot be identified, the matter must be referred to the Chief Scientist for further investigation.

Depending upon the Chief Scientist's evaluation, the analyst may or may not be required to prepare and re-run the samples again. Additionally, if the results are significantly different than the expected concentrations based on historical data or related samples, then the analyst may split the RFS sample in the laboratory and analyze the splits. Once a decision is made, full documentation of the corrective action procedures and assessment of the final result must be filed with the WQS QM Technical Lead (Marvin Palmer) or the GLNPO QM. For analyses conducted at contract or grantee laboratories, this information can be included with submitted data. When contractor or grantee laboratories have a question regarding acceptable corrective actions, they should contact the Biology Technical Lead or Limnology Technical Lead as appropriate for instruction at a time when corrective action can still be taken.

9.7 Data Reporting/Recording

- 9.7.1 When corrective actions are not feasible or do not resolve QC failure, the analyst is responsible for identifying all failed QC samples and RFS samples. If analyses are being conducted onboard, the analyst should document the QC information on the hard-copy Field Information Recording Forms (Appendix H). If analyses are being conducted by contract or grantee laboratories, the analyst should document all QC information with the submitted data.

10.0 SAFETY AND WASTE HANDLING

- 10.1 Required maintenance is described in the Lachat Procedural SOP.
- 10.2 Refer to GLNPO's *Health, Safety and Environmental Compliance Manual* (May 1997, or as amended) and individual instrument procedural operations manuals for specific details on applicable 1) personal health and safety issues; 2) instrumental, chemical, and waste handling procedures; and 3) accident prevention. This applies to all EPA personnel, EPA contractors or federal, state, or local government agencies, and persons who operate or are passengers onboard US EPA GLNPO vessels during all activities and surveys.
- 10.3 All applicable safety and waste handling rules are to be followed. These include proper labeling and disposal of chemical wastes. Over-board discharges of chemical wastes are forbidden.
- 10.4 During sampling, caution, common sense, and good judgement should dictate appropriate safety gear to be worn in any given situation on deck. Hard hats, gloves, and steel-toed shoes must be worn in working conditions where there is a possibility of injury to the head, hands, or feet; however, if in doubt, please ask the Chemical Hygiene Officer.
- 10.5 Collecting samples in cold weather, especially around cold water bodies, carries the risk of hypothermia and frostbite. Sampling team members should wear adequate clothing for protection in cold weather. For specific information regarding sampling during cold conditions, please refer to the US EPA GLNPO *Standard Operating Procedures for Winter Operations* (December 1994, or as amended).
- 10.6 Collecting samples in extremely hot and humid weather carries the risk of dehydration and heat stroke. Sampling team members should carry an adequate supply of water or other liquids for protection against dehydration in hot weather.
- 10.7 Work vests must be worn while working on the fantail and Rosette deck.

11.0 PREVENTATIVE MAINTENANCE

- 11.1 Required maintenance is described in the Lachat Procedural SOP.

12.0 TROUBLESHOOTING

- 12.1 The most common problem is deactivation of the cadmium column which result in, low values and non-linear calibration curves. A column having an efficiency factor $\leq 89\%$ quantifies the deactivation of the column.
- 12.2 The only solution is replacement of the column. This procedure is outlined in the following section.

13.0 CADMIUM COLUMN PREPARATION

NOTE: *Prepacked cadmium columns are available from Lachat Instruments.*

13.1 Preparation of Reagents for Cadmium Column

- 13.1.1 1 N Hydrochloric acid (HCl): In a 100-mL container, add 8 mL of concentrated HCl to 92 mL of reagent water. Stir to mix.
- 13.1.2 2% Copper Sulfate Solution: In a 1-L volumetric flask dissolve 20 g copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in about 800 mL of reagent water. Dilute to mark. Invert to mix thoroughly.

13.2 Cadmium Preparation and Copperization

- 13.2.1 Place 10-20 g of coarse cadmium granules (0.3-1.5 mm diameter) in a 250-mL beaker. Wash with 50 mL of acetone, then water, then two 50 mL portions of 1 N HCl. Rinse with water.
- 13.2.2 Add a 100-mL portion of 2% Copper Sulfate Solution to the cadmium prepared above. Swirl for about 5 minute, then decant the liquid and repeat with a fresh 100-mL portion of the 2% copper sulfate solution. Continue this process until the blue aqueous copper color persist. Decant and wash with at least five portions of ammonium chloride buffer to remove colloidal copper. The cadmium should be black or dark gray. The copperized cadmium granule may be stored in a stoppered bottle under ammonium chloride buffer.

13.3 Packing the Column

- 13.3.1 Wear gloves and do all cadmium transfers over a special tray or beaker. Clamp the empty column upright so that your hands are free. Unscrew one of the colored fittings from an end of the column, and pull out and save the foam plug. The column and threads are glass so be careful not to break or chip them. Fasten this fitting up higher than the open end of the column and completely fill the column, attached fittings, and tubing with ammonium chloride buffer. Scoop up prepared copperized cadmium granules with a spatula and pour them unto the top of the filled column so that they sink down to the bottom of the column. Continue pouring the cadmium in and tapping the column with a screw driver handle to dislodge any air bubbles and to prevent gaps in the cadmium filling. When the cadmium granules reach to about 5 mm from the open end of the column, push in the foam plug and screw on the top fitting. Rinse the outside of the column with reagent water.

If air remains in the column or is introduced accidentally, connect the column into the manifold, turn the pump on maximum, and tap firmly with a screwdriver handle, working up the column until all air is removed.

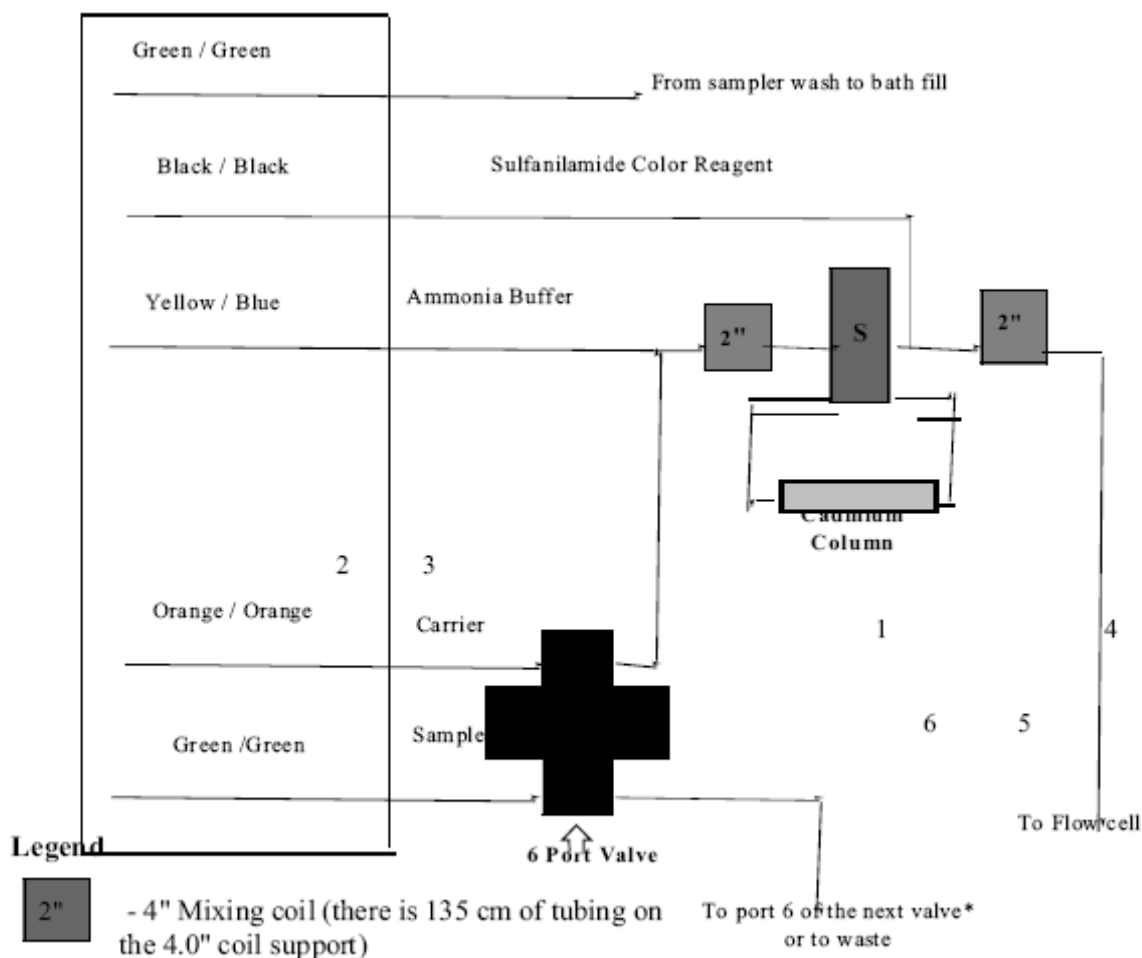
13.4 Cadmium Column Insertion Procedure

- 13.4.1 Before inserting the column, pump all reagents into manifold.
- 13.4.2 Turn the pump off.
- 13.4.3 On the column, disconnect the center tubing from one of the union connectors and immediately connect to the outlet tubing of the buffer mixing coil.
- 13.4.4 Connect the open tubing on the column to the tee fitting where the color reagent is added. **DO NOT LET AIR ENTER THE COLUMN.**
- 13.4.5 Return the pump to normal speed.
- 13.4.6 The direction of reagent flow through the column is not relevant.

14.0 REFERENCES

- 14.1 Lachat Instruments, Method Number 10-107-04-1-C, Determination of Nitrate/Nitrite in Surface and Wastewater by Flow Injection analysis. Revision Date January 1999.
- 14.2 Lachat QuikChem AE Operating Manual.

15.0 NITRATE - NITRITE ANALYTICAL MANIFOLD



S - This is a 2 state switching valve used to place the cadmium column in-lane with the manifold

Comments

1. Filter used is 520 nm.
 2. Sample loop length is 22.5 cm (0.032") ID.
 3. All manifold tubing is 0.8 mm (0.032") ID. This related to a flow of 5.2 L/cm.
 4. The **carrier** is helium degased reagent water.
- * If more than one channel is being used.